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Polyamidoamine dendrimer-conjugated quantum dots for efficient labeling of primary cultured mesenchymal stem cells

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ABSTRACT

Monitoring of cells *in vivo* after transplantation could supply important information for determining the efficacy of stem cell therapy. The use of quantum dots (QDs) has several advantages for *in vivo* imaging, such as remarkable resistance to photo bleaching, high fluorescence efficiency, and size-tunable emission. After they are taken up by cells via endocytosis, QDs lose their fluorescence intensity in endosomes/lysosomes at low pH because the intensity cannot survive under acidic conditions. Moreover, the amount of QD uptake by mesenchymal stem cells (MSCs) is extremely small. Therefore, for effective labeling of MSCs and long observation of MSCs labeled by QDs *in vivo*, it is essential both to increase cellular uptake of QDs and to promote endosomal escape into the cytosol. The polyamidoamine (PAMAM) dendrimer had plenty of cationic charge, which promoted cellular uptake through electrostatic interactions, and a “buffering capacity,” which enhanced endosomal escape into the cytosol. In this study, QDs were modified with PAMAM dendrimer for the efficient labeling of MSCs by QDs. The uptake efficiency and cytosolic distribution of QDs in primary cultured MSCs were increased by the modification of the PAMAM dendrimer. The fluorescence intensity in MSCs labeled by PAMAM dendrimer-conjugated QDs lasted for a longer time in harvested culture plates or in cell-transplanted mice than that in MSCs labeled by non-conjugated QDs.

Key words:

quantum dot, stem cell, *in vivo* imaging, PAMAM dendrimer, endosomal escape, buffering effect

1. Introduction

Mesenchymal stem cells (MSCs) are multipotent stem cells that have generated a great deal of excitement as a potential source of cell-based therapies [1,2]. Recently, MSCs have been used clinically for several diseases, such as amyotrophic lateral sclerosis [3], acute myocardial infarction [4], liver cirrhosis [5], and Parkinson's disease [6]. The rational design of cell based-therapies requires monitoring of cell survival and location after transplantation in the host organism. In order to evaluate the distribution, survival and function of transplanted stem cells after administration, several methods for labeling stem cells have been developed, such as optical imaging, radionucleotide-based imaging and magnetic resonance imaging (MRI) [7,8]. Although MRI can reveal the 3-dimensional anatomy in excellent detail, its low sensitivity makes the detection of smaller numbers of cells technically difficult in living animal [9,10]. Radionucleotide-based imaging methods include positron emission tomography (PET) and single-photon emission computed tomography (SPECT).

These methods can also provide 3-dimensional information and have relatively high resolution. However, they lack anatomical information and require exposure to high levels of radiation [11,12].

Compared to these imaging approaches, optical imaging involving bioluminescence and fluorescence shows higher sensitivity, although it is subject to certain limitations with respect to 3-dimensional information. Recently, great advances have been made in fluorescence imaging techniques, enabling single-cell detection and compatibility with conventional microscopy [13]. Quantum dots (QDs) are a new generation of inorganic probes that consist of semiconductor nanocrystals. Compared with traditional fluorophores such as GFP, FITC, and rhodamine, QDs possess tremendous advantages due to their optical properties for cellular labeling and imaging, remarkable resistance to photo bleaching, high fluorescence efficiency, size-tunable emission, broader absorption spectra and narrower emission spectra [14,15]. Because of the stability and brightness of QDs, several studies have examined their potential for *in vivo* imaging of tumor cells [16] and stem cells in live animals [17,18]. However, the lower cellular uptake of QDs would constitute a major obstacle for *in vivo* fluorescent imaging. Another problem is that QDs are trapped in endosomes/lysosomes after endocytosis. Because the fluorescence of the QD decreases as the pH decreases, the fluorescence intensity of QDs would be decreased non-reversibly after QDs move into endosomes/lysosomes with a pH of around 4.0-5.0 in the cells [19-21]. In order to use QDs for labeling stem cells with strong fluorescence for a long period, it is essential to enhance both the cellular uptake and endosomal escape of QDs.

Cationic polymers with a large number of primary amine groups, such as polyamidoamine (PAMAM) dendrimer, and grafted polyethylene imine (PEI), have been widely used to promote cellular uptake through the electrostatic interaction between their positive charge and the negative charge on the cell membrane in the development of gene delivery carriers [22,23]. Furthermore, these polymers have a strong pH-buffering capacity that could enhance proton absorption in acid organelles and an osmotic pressure buildup across the organelle membrane. These processes in turn could promote endosomal escape and release into the cytoplasm [24,25]. Recently, Duan et al. have demonstrated that QDs coated with PEI-PEG conjugates were spread throughout the cytoplasm after internalization by endocytosis in a human epithelial carcinoma cell line, Hela cells [26]. Modification of QDs by cationic polymers with buffering capacity is expected to enhance the cytoplasmic migration of QDs in MSCs through the acceleration of endosomal escape, which prevents the decrease of fluorescence intensity of QDs. However, little has been done to clarify that cytosolic delivery could retain fluorescence intensity of QDs in cells.

The objective of this article is to label MSCs by QDs for *in vivo* imaging. PAMAM dendrimers are unique highly branched spherical polymers with a narrow size distribution, and have strong buffering capacity [27]. Here, we synthesized QDs

modified with PAMAM dendrimer to enhance both the cellular uptake and endosomal escape in MSCs that were isolated from mice bone marrow. The cellular uptake efficiency, intracellular distribution, and cytotoxicity of PAMAM dendrimer-conjugated QDs were investigated. Furthermore, the biodistribution and fluorescence intensity of MSCs labeled with PAMAM dendrimer-conjugated QDs were examined in mice.

2. Material and methods

2.1. Materials

Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum, NH₂-PEG_{2,000}-QD, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid, sulfosuccinimidyl ester, sodium salt (BODIPY[®] FL, SSE) and transferrin-Alexa Fluor[®] 488 conjugate were purchased from Invitrogen (Carlsbad, CA). PAMAM dendrimer generation 4.0 was purchased from Sigma-Aldrich (St.Louis, MO). Bis [sulfosuccinimidyl] suberate (BS₃) was purchased from Thermo Fisher Scientific (Waltham, MA). Vivaspın, NAP-5 column and Sephadex G25 was purchased from GE Healthcare UK Ltd. (Buckinghamshire, England).

2.2. Animals

Male six-week-old C57BL6 mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals. All animal experiments were carried out in accordance with the Principles of Laboratory Animal Care as adopted and promulgated by the US National Institutes of Health and by the Guidelines for Animal Experiments of Kyoto University

2.3. Isolation and culture of MSCs

The isolation method was performed as described by Yagi et al. [28]. Briefly, bone marrow cells were isolated from mice by flushing the femurs with DMEM containing 10% fetal bovine serum, 1000U/ml penicillin G, 100 mg/l streptomycin, 100 µg/l amphotericin B, and 100 µg/l aprotinin using a 25-gauge needle. Isolated cells were seeded onto 24-well dishes at a density of 3×10^6 cells/cm² and cultured for 9 days in DMEM at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. The culture medium was replaced every two days to remove the unadherent cells. The plastic-adherent cells after 9 days were used for the experiment.

2.4. Synthesis of PAMAM dendrimer-conjugated QDs

QDs (8 μ M) were added to the BS_3 solution (final concentration: 0.5 mM). After rotating for 1 h at room temperature, the solution was purified through a desalting NAP-5 column. PAMAM dendrimer was added to the collected solution at a molar ratio of 40: 1 (PAMAM dendrimer: o QDs). The solution was mixed gently and rotated for 2 h at room temperature. The reaction was quenched with 1 M glycine by adding it to a final concentration of about 50 mM for 15 minutes. The conjugate was purified by Sephadex 25 gel-filtration by PBS, pH 7.4. The number of amino groups was determined by trinitrobenzenesulfonic acid (TNBS) methods and fluorescent probe labeling methods. In briefly, 0.5 mg PAMAM dendrimer was reacted with 0.042 mg BODIPY in 165 μ l NaHCO_3 (100 mM) for 12 h at 4 $^\circ\text{C}$. After free BODIPY was removed by Vivaspin (5000MWCO), the number of BODIPY conjugated on PAMAM dendrimer was determined by TNBS method and molar extinction coefficient of BODIPY at 504nm. An average BODIPY/PAMAM ratio was 2.03. BODIPY-PAPAM-QD was synthesized, then the number of PAMAM-BODIPY on QD was calculated by the molar extinction coefficient of 350nm (QD) and 504nm (BODIPY).

2.5. *Measurement of zeta potential, photoluminescence spectra and fluorescence intensity*

The zeta potential of 0.016 μ M PAMAM dendrimer-conjugated QDs and non-conjugated QDs in water solution was measured by Zeta Sizer Nano-ZS (Malvern Instruments, Ltd., Worcestershire, UK). The photoluminescence spectra were recorded on an RF-540 Spectro fluorophotometer (Shimadzu Co., Kyoto, Japan). The fluorescence intensity of 200 μ l of QDs dispersion was measured by multilabel counter ARVOTM mx 1420 (ParkinElmer, Boston, MA).

2.6. *Cellular uptake and imaging*

MSCs were cultured with DMEM containing PAMAM dendrimer-conjugated QDs or non-conjugated QDs. The MSCs were trypsinized and washed with PBS three times, and then fluorescence intensity was analyzed using a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ). For microscopic images, MSCs were cultured with DMEM containing PAMAM dendrimer-conjugated QDs or non-conjugated QDs on a cover glass on culture plates. Cells fixed with 4% paraformaldehyde were stained with DAPI and mounted with glycerin solution. The MSCs were observed with a BZ-8100 fluorescence microscope (Keyence Co., Osaka, Japan). For the analysis of intracellular localization, 10 μ l of transferrin-Alexa Fluor® 488 conjugates (5 mg/ml in PBS) was added to the DMEM containing QDs before application to the MSCs. To inhibit H^+ pump function, 10 μ l of bafilomycin A (10 mM) was mixed with the DMEM

containing QDs before addition to the cell culture.

2.7. *Measurement of cytotoxicity*

Cytotoxicity was evaluated by the standard method of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, MTT solution was added to MSCs after they were cultured with QDs or PAMAM dendrimer-QD conjugates. After incubating for 4 h, UV-vis absorption was measured at 550 nm with 655 nm as the reference wavelength using a Model 550 Microplate Reader (Bio-Rad Laboratories Inc., Hercules, CA).

2.8. *Transplantation of MSCs into mice*

A suspension of 5×10^5 labeled stem cells in 0.3 ml PBS was injected into the tail veins of mice. After the mice were killed, the lungs, spleen, liver and other organs were removed and washed three times with PBS. Collected tissues were fixed in 10% formalin at room temperature for 15 min and then washed three times for 10 min in PBS on ice. The organs were suspended in 10% sucrose-PBS for 2 h and then in 20% sucrose-PBS overnight at 4°C. Then, the tissues were frozen in liquid nitrogen and cryosectioned at 10- μ m thickness. Fifteen sections were chosen at random, and the numbers of PAMAM dendrimer-conjugated QDs or QD-labeled cells were counted in an area of 2465 μ m \times 2303 μ m in fluorescence microscopic images.

3. Results

3.1. *Characterization*

The zeta potential of QDs and PAMAM dendrimer-conjugated QDs were - 19.3 mV and + 2.76 mV, respectively. The average number of PAMAM dendrimers conjugated with QDs was calculated by the number of surface amino groups. There were 12.8 PAMAM dendrimers modified per QD. The fluorescence spectra of PAMAM dendrimer-conjugated QDs was similar to that of the QDs themselves (Figure 1).

3.2. *Cellular accumulation*

After MSCs were cultured with PAMAM dendrimer-conjugated QDs or QDs for 1 h at 10 nM, weak intracellular fluorescence was observed in the cells treated with PAMAM dendrimer-conjugated QDs, although no fluorescence signals were detected in the cells treated with non-conjugated QDs (Figure 2A, 2B). After 13 h of incubation, strong fluorescence signals were observed in almost all of the cells treated with PAMAM

dendrimer-conjugated QDs, although only weak fluorescence signals were observed in some of the cells treated with non-conjugated QDs (Figure 2C, 2D). The cellular uptake of PAMAM dendrimer-QD conjugates was increased depending on the concentration of QDs and the incubation period (Figure 2E). After 24 h of incubation, the intracellular fluorescence of PAMAM dendrimer-conjugated QDs was 70-80 times stronger than that of the non-conjugated QDs (Figure 2E).

3.3. *Endosomal escape*

In order to compare the intracellular distribution between PAMAM dendrimer-conjugated QDs and non-conjugated QDs (red), endosomes were labeled with transferrin-Alexa Fluor 488 conjugates (green). After the QDs were engulfed into the cells, their intracellular distribution was almost coincident with that of transferrin-Alexa Fluor 488 (Figure 4A). This result suggested that most of the QDs were entrapped within endosomes. On the other hand, the fluorescence signals of the PAMAM dendrimer-conjugated QDs were partially observed outside endosomes, suggesting that PAMAM dendrimer-conjugated QDs partially escaped from the endosome. When a molecule possessing a strong buffering capacity is entrapped in the endosome, a large H^+ charge flows into the endosome through a proton pump [24,25]. In order to investigate the effect of the buffering capacity of the PAMAM dendrimer on endosomal escape, the intracellular distribution of the PAMAM dendrimer-conjugated QDs was investigated with a proton pump inhibitor, bafilomycin A. The distribution of PAMAM dendrimer-conjugated QDs was coincident with the distribution of endosome when PAMAM dendrimer-conjugated QDs were incubated with bafilomycin A (Figure 3C).

3.4. *Retention of fluorescence signals in cultured cells*

Primary cultured MSCs were labeled at approximately the same fluorescence intensity with PAMAM dendrimer-conjugated QDs (0.625 nM, 24 h) or non-conjugated QDs (20 nM, 48 h). After labeling MSCs, the culture medium was changed to DMEM without PAMAM dendrimer-conjugated QDs or without non-conjugated QDs. Although the fluorescence signals in cells were significantly decreased in the cells labeled with non-conjugated QDs (Figure 4A), the signals were only slightly decreased in the case of PAMAM dendrimer-conjugated QDs on the 3rd day after changing the culture medium to QD-free medium (Figure 4B). Significant cellular toxicity was not observed at the lower concentration of 40 nM after labeling with either PAMAM dendrimer-conjugated QDs (for 48 h) or non-conjugated QDs (for 72 h) (Figure 5).

3.5. *Retention of fluorescence signals in mice*

MSCs labeled at approximately the same fluorescence intensity with PAMAM dendrimer-conjugated QDs (0.625 nM, 24 h) or non-conjugated QDs (20 nM, 48 h) were injected into mice. Frozen sections of the lung, liver and spleen at 6 h after injection are shown in Figure 6. A large number of cells with fluorescent signals of QDs were observed in the lung but not in the liver or spleen (data not shown). The number of cells with fluorescent signals of QDs rapidly decreased in the lung but increased in the liver and in the spleen at 6 h (data not shown). Then, we evaluated the number of cells with fluorescent signals of QDs in the liver and spleen. The number of cells with fluorescent signals was decreased in the case of cells labeled with non-conjugated QDs (Figure 7A). In contrast, the number of cells with fluorescent signals remained unchanged in the case of cells labeled with PAMAM dendrimer-conjugated QDs (Figure 7B).

4. Discussion

The two major purposes of this study were to enhance cellular accumulation of QDs and to enhance endosomal escape of QDs for the maintenance of strong fluorescence intensity of QDs. Previously, we and another group have modified QDs with several types of ligands, such as antibodies [29] and sugars [30], to enhance the cellular uptake via specific interactions with the target cells. Because MSCs expressed no molecule which would be useful for specific uptake for target cells, a method using an electrostatic interaction to enhance cellular uptake would be effective. PAMAM dendrimer is an attractive carrier for nucleic acids and pDNA with a substantial number of amino groups which could provide positive charges and strong buffering capacity [26]. pDNA or oligonucleotides complexed with PAMAM dendrimer could be effectively taken up by cells via electrostatic interaction [22,23]. Moreover, Sonawane et al. have demonstrated that PAMAM dendrimer complexed with pDNA enhanced the concentration of Cl^- and prevented the decrease of pH in the endosome [24,25]. Therefore, we synthesized PAMAM dendrimer-conjugated QDs. The intensity of PAMAM dendrimer-conjugated QDs was similar to that with non-conjugated QDs, suggesting that fluorescence intensity was not influenced by modification of QDs with PAMAM dendrimer. The zeta potential is usually measured as an indicator of surface charge of nanoparticles. In this study, the zeta potential of PAMAM dendrimer-conjugated QDs was slightly positive (+ 2.76 mV), although the zeta potential of QDs was negative (- 19.3 mV). Moreover, the cellular uptake of PAMAM dendrimer-conjugated QDs was significantly higher than that of non-conjugated QDs (Fig. 2). These results indicated that modification of QDs with PAMAM dendrimer could enhance cellular uptake through the non-specific interaction with primary cultured

MSCs.

In order to investigate endosomal escape of QDs or PAMAM dendrimer-conjugated QDs, the intracellular distributions of QDs or PAMAM dendrimer-conjugated QDs were observed by fluorescent microscopy (Fig. 3). Almost all QDs were entrapped in the endosomes/lysosomes (Fig. 3). Similar results were observed in QDs that were incubated with vero cells [31]- and T cells [32]. On the other hand, PAMAM dendrimer-conjugated QDs were partially escaped from endosomes/lysosomes and spread throughout the cytosol (Fig. 3). In order to investigate the mechanism of endosomal escape, we additionally performed a comparison of the intracellular distribution of PAMAM dendrimer-conjugated QDs with or without bafilomycin A1, which prevents endosomal acidification by inhibiting the vacuolar H⁺-ATPase endosomal proton pump [33,34]. Bafilomycin A1 significantly suppressed the endosomal escape of pDNA or oligonucleotide complexed with PEI [35]. In this study, the endosomal escape of PAMAM dendrimer-conjugated QDs was inhibited by bafilomycin A1 (Fig. 3). These results suggest that protonation of PAMAM dendrimer in endosomes/lysosomes would promote endosomal escape of PAMAM dendrimer-conjugated QDs into the cytosol in primary cultured MSCs.

When measuring the duration of fluorescence labeling, the initial fluorescence intensity of MSCs labeled with non-conjugated QDs must be similar to that of MSCs labeled with PAMAM dendrimer-conjugated QDs. However, PAMAM dendrimer-conjugated QDs were shown to label MSCs more effectively than non-conjugated QDs. Therefore, the labeling condition for MSCs was selected as 0.625 nM, 24 h for PAMAM dendrimer-conjugated QDs or 20 nM, 48 h for non-conjugated QDs in order to adjust the initial fluorescence intensity of MSCs labeled with PAMAM dendrimer-conjugated QDs or non-conjugated QDs (Fig. 4). Although the fluorescence intensity of MSCs labeled with QDs was rapidly decreased and almost entirely gone at 72 h, the fluorescence intensity of MSCs labeled with PAMAM dendrimer-conjugated QDs was maintained at 48 h and little decreased at 72 h (Fig 4). These results are compatible with the results shown in Fig. 7, in which fluorescent signals in the liver and spleen of mice were maintained in MSCs labeled with PAMAM dendrimer-conjugated QDs but not in those labeled with non-conjugated QDs at 24 h after intravenous injection of labeled MSCs in mice (Fig. 7). Kraitchman et al. demonstrated that MSCs labeled with radiotracer and MR contrast agent could be detected in liver and spleen within 24 - 48 h after intravenous injection in mice [36]. Therefore, the decrease in intensity of fluorescence signals in the liver and spleen after intravenous injection of MSCs labeled with non-conjugated QD might represent a decrease in the fluorescence intensity of QDs but not the disappearance of MSCs. Previous reports have demonstrated that the acidic environment would detach surface-coating molecules and lead to the destruction and oxidization of QDs, which would decrease the fluorescence intensity [19-21]. We also observed that the

fluorescence intensity of QDs in the solution at pH 3 had decreased to 20% of the initial fluorescence intensity at 72 h, although the fluorescence intensity of QDs in the solution at pH 7 at 72 h was at least 80% of the initial fluorescence intensity (data not shown). Take these facts into consideration, MSCs labeled by PAMAM dendrimer-conjugated QDs would maintain their fluorescence intensity via endosomal escape into the cytosol under a neutral pH in primary cultured MSCs.

Several types of agents, such as synthesized polymers, virus-derived peptides or proteins, could enhance endosomal escape through a pH buffering effect, pore formation in the endosomal membrane, or fusion in the endosomal membrane [37]. These agents could be candidate molecules for prolongation of the fluorescence intensity of endocytosed QDs in cells. However, some of them cannot be used for the modification of QDs because of their strong cytotoxicity and influence on the cell function. Previously, we and other groups demonstrated that a dendron/dendrimer that consists of amino acids with a buffering effect, such as lysine or histidine, could enhance the endosomal escape of pDNA or oligonucleotides [38,39]. A dendron/dendrimer composed of lysine or histidine would be a promising tool for the modification of QDs with little toxicity.

5. Conclusion

The modification of QDs with PAMAM dendrimer promoted the uptake of QD into primary cultured MSCs through electrostatic interaction. After PAMAM dendrimer-conjugated QDs were taken up by primary cultured MSCs via endocytosis, PAMAM dendrimer-conjugated QDs were distributed throughout the cytoplasm through the buffering ability of the conjugated PAMAM dendrimer. Moreover, this cytosolic delivery contributed to prolongation of the intracellular fluorescence intensity in primary cultured MSCs both *in vitro* and *in vivo*.

Acknowledgements

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Figure legends

Figure 1

Fluorecence intensity of QDs (open circle) and PAMAM dendrimer-conjugated QDs (closed circle).

Figure 2

Cellular uptake of QDs and PAMAM dendrimer-conjugated QDs in primary cultured MSCs. Cells were incubated with PAMAM dendrimer-conjugated QDs (10 nM) or QDs (10 nM) for 1 and 13 h. After cells were fixed with 4% paraformaldehyde, cells were observed by fluorescent microscopy (A). Cells were incubated with PAMAM dendrimer-conjugated QDs for 12 h (open circle) or 24 h (closed circle), and QDs for 12 h (closed triangle) at the indicated concentration. Fluorescence intensity was measured by flow cytometry (B).

Figure 3

Intracellular distribution of QDs and PAMAM dendrimer-conjugated QDs in primary cultured MSCs. Cells were incubated with QDs (red) (50 nM) without bafilomycin A (A), with PAMAM dendrimer-conjugated QDs (red) (1 nM) and bafilomycin A (B), and with PAMAM dendrimer-conjugated QDs (red) (1 nM) without bafilomycin A (C). Endosomes were stained with transferrin-Alexa Fluor® 488 conjugates (green).

Figure 4

Retention of the fluorescent signals of non-conjugated QDs and PAMAM dendrimer-conjugated QDs in primary cultured MSCs. Cells were incubated with non-conjugated QDs (red) for 48 h at 20 nM (A) or PAMAM dendrimer-conjugated QDs (red) for 24 h at 0.625 nM (B), and the media were exchanged for culture medium without non-conjugated QDs or without PAMAM dendrimer-conjugated QDs and cultured for the indicated number of days. Nuclei were stained with DAPI (blue).

Figure 5

Cytotoxicity of PAMAM dendrimer-conjugated QDs in primary cultured MSCs. Cell viability was investigated by MTT assay after cells were incubated with PAMAM dendrimer-conjugated QDs for 24 h (closed circle) or 48 h (open circle) at 2.5, 5.0, 10 or 40 nM.

Figure 6

In vivo distribution of primary cultured MSCs labeled with QDs or PAMAM dendrimer-conjugated QDs. Cells were incubated with QDs (red) (A) or PAMAM dendrimer-conjugated QDs (red) (B) for 24 h at 0.625 nM. After trypsinization, cells (5.0×10^5 cells/mouse) were intravenously injected into the tail vein of mice. After 6 h, the lung, liver and spleen were excised from mice and frozen sections were prepared. Nuclei were stained with DAPI (blue).

Figure 7

Retention of the fluorescent signals of QDs and PAMAM dendrimer-conjugated QDs in mice. MSCs were incubated with QDs (red) for 48 h at 20 nM (A) or PAMAM dendrimer-conjugated QDs (red) for 24 h at 0.625 nM (B). After trypsinization, MSCs (5.0×10^5 cells/mouse) were intravenously injected into the tail vein of mice. After 6 h, the liver (A) and spleen (B) were excised from mice and frozen sections were prepared. Nuclei were stained with DAPI (blue). The numbers of cells that were labeled with QDs or PAMAM dendrimer-conjugated QDs were counted.

Fig.1

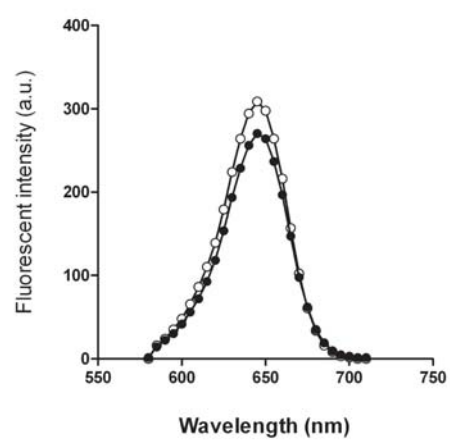
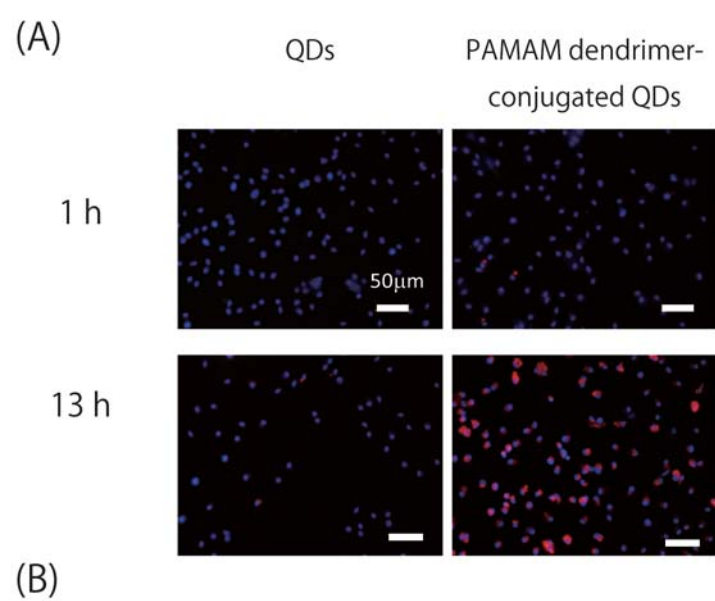


Fig.2



(B)

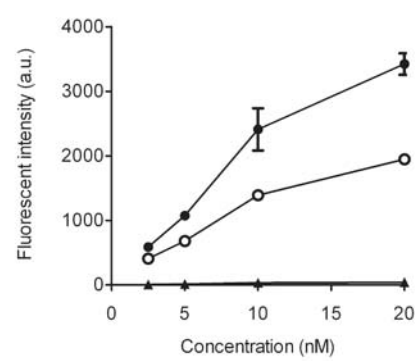
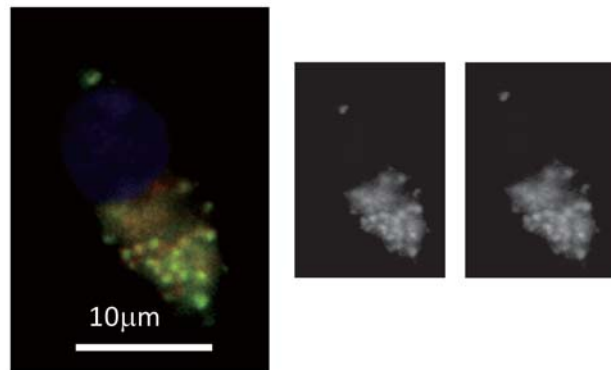
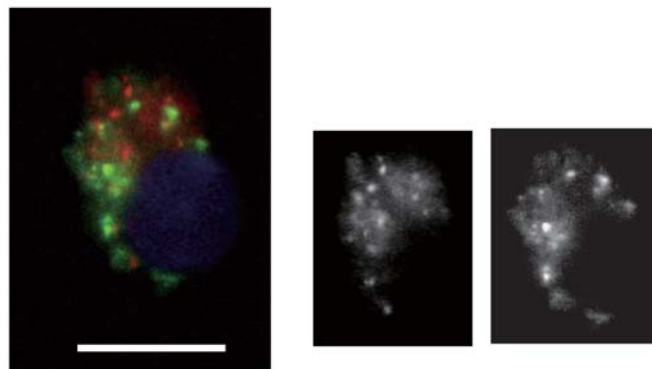


Fig.3

(A) QDs (50 nM) without Bfilomycin A



(B) PAMAM dendrimer-conjugated QDs (1 nM) without Bfilomycin A



(C) PAMAM dendrimer-conjugated QDs (1 nM) with Bfilomycin A

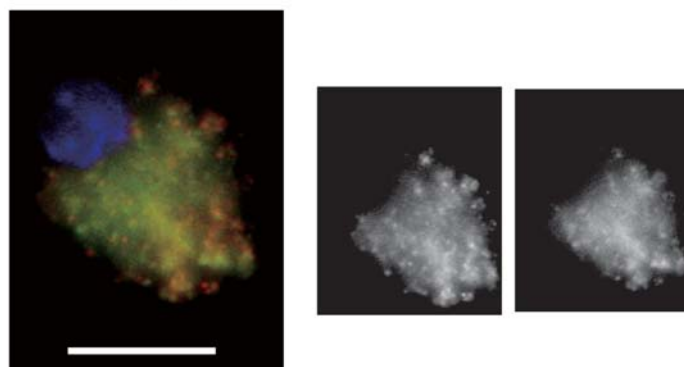


Fig.4

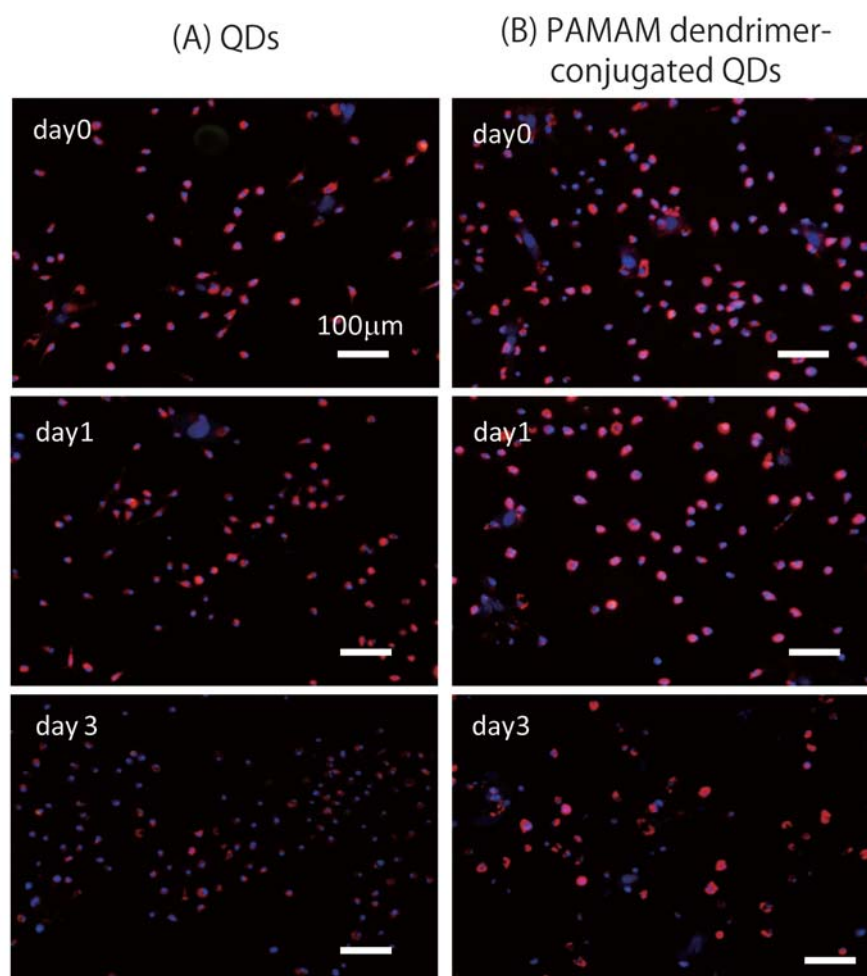


Fig.5

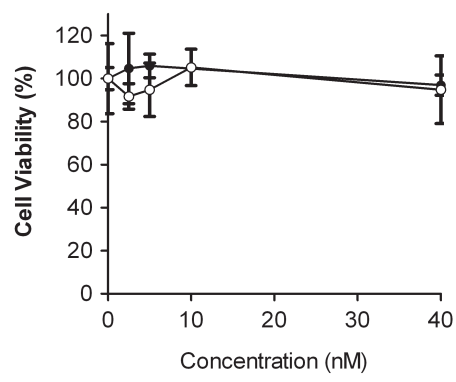


Fig.6

(A) QDs

(B) PAMAM dendrimer-
conjugated QDs

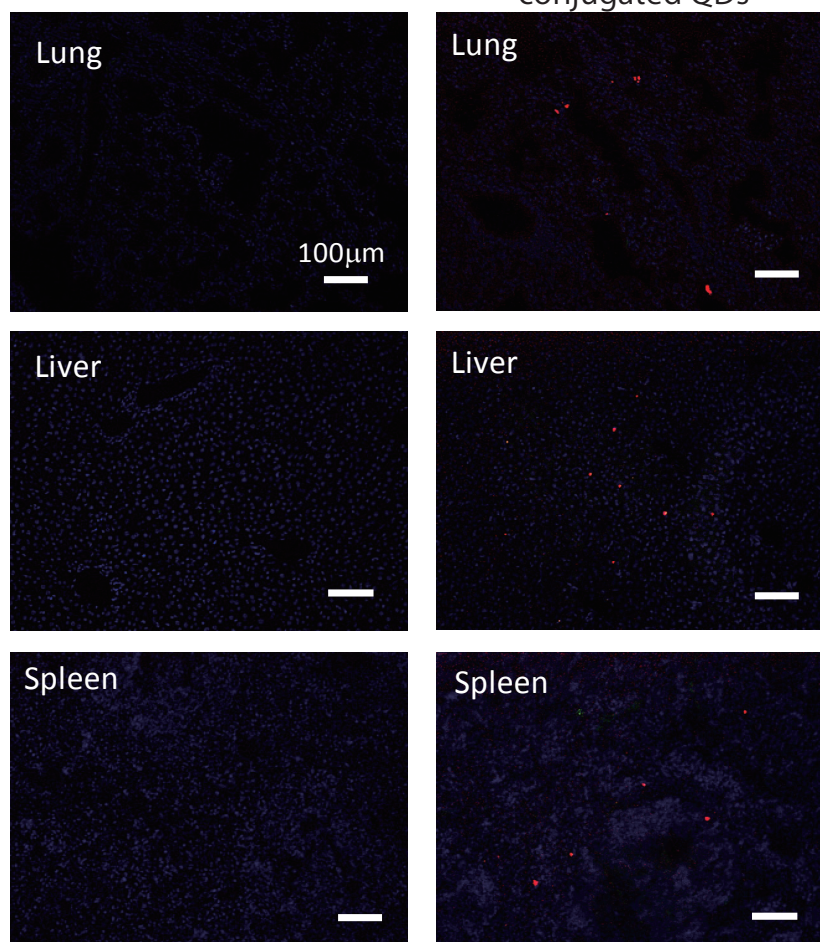
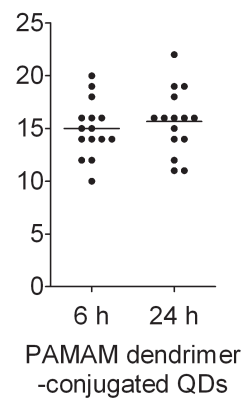
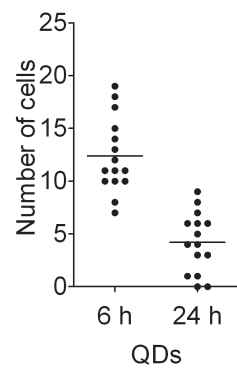


Fig. 7

(A) Liver



(B) Spleen

